EUROPEAN PATENT APPLICATION

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㊺ Recombinant antibodies and methods for their production.

㊿ An altered antibody is produced by replacing the complementarity determining regions (CDRs) of a variable region of an immunoglobulin (Ig) with the CDRs from an Ig of different specificity, using recombinant DNA techniques. The gene coding sequences for producing the altered antibody may be produced by site-directed mutagenesis using long oligonucleotides.
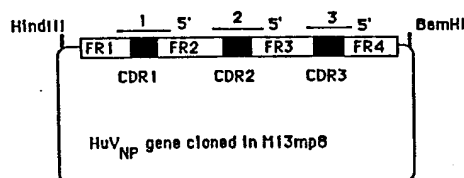
HindIII ⎍ 1 5' 2 5' 3 5' BamHI

FR1 | CDR1 | FR2 | CDR2 | FR3 | CDR3 | FR4

HuV_NP gene cloned in M13mp8

D1.3 CDR1 oligonucleotide
5' CTG,TCT,CAC,CCA,GTT,TAC,ACC,ATA,GCC,GCT,GAA,GGT,GCT

FR2   D1.3 CDR1   FR1

D1.3 CDR2 oligonucleotide
5' CAT,TGT,CAC,TCT,GGA,TTT,GAG,AGC,TGA,ATT,ATA,GTC,TGT,

FR3   D1.3 CDR2

GTT,TCC,ATC,ACC,CCA,AAT,CAT,TCC,AAT,CCA,CTC

D1.3 CDR2   FR2

D1.3 CDR3 oligonucleotide
5' GCC,TTG,ACC,CCA,GTA,GTC,AAG,CCT,ATA,ATC,TCT,CTC,TCT,

FR4   D1.3 CDR3

TGC,ACA,ATA

FR3

RECOMBINANT DNA PRODUCT AND METHODS

The present invention relates to altered antibodies
in which at least parts of the complementarity
determining regions (CDRs) in the light or heavy
chain variable domains of the antibody have been
replaced by analogous parts of CDRs from an antibody
of different specificity. The present invention
also relates to methods for the production of such
altered antibodies.

Natural antibodies, or immunoglobulins, comprise two
heavy chains linked together by disulphide bonds and
two light chains, one light chain being linked to
each of the heavy chains by disulphide bonds. The
general structure of an antibody of class IgG (i.e.
an immunoglobulin (Ig) of class gamma (G)) is shown
schematically in Figure 1 of the accompanying
drawings.

Each heavy chain has at one end a variable domain
followed by a number of constant domains. Each
light chain has a variable domain at one end and a
constant domain at its other end, the variable
domain being aligned with the variable domain of the
heavy chain and the constant domain being aligned
with the first constant domain of the heavy chain.
The constant domains in the light and heavy chains
are not involved directly in binding the antibody to
the antigen.

The variable domains of each pair of light and heavy
chains form the antigen binding site. The domains
on the light and heavy chains have the same general
structure and each domain comprises four framework

regions, whose sequences are relatively conserved,
connected by three hypervariable or complementarity
determining regions (CDRs) (see Kabat, E.A., Wu,
T.T., Bilofsky, H., Reid-Miller, M. and Perry, H.,
in "Sequences of Proteins of Immunological
Interest", US Dept. Health and Human Services 1983).
The four framework regions largely adopt a β-sheet
conformation and the CDRs form loops connecting, and
in some cases forming part of, the β-sheet
structure. The CDRs are held in close proximity by
the framework regions and, with the CDRs from the
other domain, contribute to the formation of the
antigen binding site.

For a more detailed account of the structure of
variable domains, reference may be made to: Poljak,
R.J., Amzel, L.M., Avey, H.P., Chen, B.L.,
Phizackerly, R.P. and Saul, F., PNAS USA, 70,
3305-3310, 1973; Segal, D.M., Padlan, E.A., Cohen,
G.H., Rudikoff, S., Potter, M. and Davies, D.R.,
PNAS USA, 71, 4298-4302, 1974; and Marquart, M.,
Deisenhofer, J., Huber, R. and Palm, W., J. Mol.
Biol., 141, 369-391, 1980.

In recent years advances in molecular biology based
on recombinant DNA techniques have provided
processes for the production of a wide range of
heterologous polypeptides by transformation of host
cells with heterologous DNA sequences which code for
the production of the desired products.

EP-A-0 088 994 (Schering Corporation) proposes the
construction of recombinant DNA vectors comprising a
ds DNA sequence which codes for a variable domain of
a light or a heavy chain of an Ig specific for a

predetermined ligand. The ds DNA sequence is
provided with initiation and termination codons at
its 5'- and 3'- termini respectively, but lacks any
nucleotides coding for amino acids superfluous to
the variable domain. The ds DNA sequence is used to
transform bacterial cells. The application does not
contemplate variations in the sequence of the
variable domain.

EP-A-1 102 634 (Takeda Chemical Industries Limited)
describes the cloning and expression in bacterial
host organisms of genes coding for the whole or a
part of human IgE heavy chain polypeptide, but does
not contemplate variations in the sequence of the
polypeptide.

EP-A-0 125 023 (Genentech Inc.) proposes the use of
recombinant DNA techniques in bacterial cells to
produce Ig's which are analogous to those normally
found in vertebrate systems and to take advantage of
the gene modification techniques proposed therein to
construct chimeric Igs or other modified forms of Ig.

The term 'chimeric antibody' is used to describe a
protein comprising at least the antigen binding
portion of an immunoglobulin molecule (Ig) attached
by peptide linkage to at least part of another
protein.

It is believed that the proposals set out in the
above Genentech application did not lead to the
expression of any significant quantities of Ig
polypeptide chains, nor to the production of Ig
activity, nor to the secretion and assembly of the
chains into the desired chimeric Igs.

The production of monoclonal antibodies was first
disclosed by Kohler and Milstein (Kohler, G. and
Milstein, C., Nature, 256, 495-497, 1975). Such
monoclonal antibodies have found widespread use not
only as diagnostic reagents (see, for example,
'Immunology for the 80s, Eds. Voller, A., Bartlett,
A., and Bidwell, D., MTP Press, Lancaster, 1981) but
also in therapy (see, for example, Ritz, J. and
Schlossman, S.F., Blood, 59, 1-11, 1982).

The recent emergence of techniques allowing the
stable introduction of Ig gene DNA into myeloma
cells (see, for example, Oi, V.T., Morrison, S.L.,
Herzenberg, L.A. and Berg, P., PNAS USA, 80,
825-829, 1983; Neuberger, M.S., EMBO J., 2,
1373-1378, 1983; and Ochi, T., Hawley, R.G., Hawley,
T., Schulman, M.J., Traunecker, A., Kohler, G. and
Hozumi, N., PNAS USA, 80, 6351-6355, 1983), has
opened up the possibility of using in vitro
mutagenesis and DNA transfection to construct
recombinant Igs possessing novel properties.

However, it is known that the function of an Ig
molecule is dependent on its three dimensional
structure, which in turn is dependent on its primary
amino acid sequence. Thus, changing the amino acid
sequence of an Ig may adversely affect its
activity. Moreover, a change in the DNA sequence
coding for the Ig may affect the ability of the cell
containing the DNA sequence to express, secrete or
assemble the Ig.

It is therefore not at all clear that it will be
possible to produce functional altered antibodies by
recombinant DNA techniques.

However, colleagues of the present Inventor have devised a process whereby chimeric antibodies in which both parts of the protein are functional can be secreted. The process, which is disclosed in International Patent Application No. PCT/GB85/00392 (Neuberger et al. and Celltech Limited), comprises:

a) preparing a replicable expression vector including a suitable promoter operably linked to a DNA sequence comprising a first part which encodes at least the variable domain of the heavy or light chain of an Ig molecule and a second part which encodes at least part of a second protein;

b) if necessary, preparing a replicable expression vector including a suitable promoter operably linked to a DNA sequence which encodes at least the variable domain of a complementary light or heavy chain respectively of an Ig molecule;

c) transforming an immortalised mammalian cell line with the or both prepared vectors; and

d) culturing said transformed cell line to produce a chimeric antibody.

The second part of the DNA sequence may encode:

  i) at least part, for instance the constant domain of a heavy chain, of an Ig molecule of different species, class or subclass;

  ii) at least the active portion or all of an enzyme;

  iii) a protein having a known binding specificity;

  iv) a protein expressed by a known gene but whose sequence, function or antigenicity is not known; or

  v) a protein toxin, such as ricin.

The above Neuberger application only shows the production of chimeric antibodies in which complete variable domains are coded for by the first part of the DNA sequence. It does not show any chimeric antibodies in which the sequence of the variable domain has been altered.

The present invention, in a first aspect, provides an altered antibody in which at least parts of the CDRs in the light or heavy chain variable domains have been replaced by analogous parts of CDRs from an antibody of different specificity

The determination as to what constitutes a CDR and what constitutes a framework region was made on the basis of the amino-acid sequences of a number of Igs. However, from the three dimensional structure of a number of Igs it is apparent that the antigen binding site of an Ig variable domain comprises three looped regions supported on sheet-like structures. The loop regions do not correspond

exactly to the CDRs, although in general there is considerable overlap.

Moreover, not all of the amino-acid residues in the loop regions are solvent accessible and in one case, amino-acid residues in the framework regions are involved in antigen binding.(Amit, A.G., Mariuzza, R.A., Phillips, S.E.V. and Poljak, R.J., Science, 233, 747-753, 1986).

It is also known that the variable regions of the two parts of an antigen binding site are held in the correct orientation by inter-chain non-covalent interactions. These may involve amino-acid residues within the CDRs.

Thus, in order to transfer the antigen binding capacity of one variable domain to another, it may not be necessary to replace all of the CDRs with the complete CDRs from the donor variable region. It may be necessary only to transfer those residues which are accessible from the antigen binding site, and this may involve transferring framework region residues as well as CDR residues.

It may also be necessary to ensure that residues essential for inter-chain interactions are preserved in the acceptor variable domain.

Within a domain, the packing together and orientation of the two disulphide bonded β-sheets (and therefore the ends of the CDR loops) are relatively conserved. However, small shifts in packing and orientation of these β-sheets do occur

(Lesk, A.M. and Chothia, C., J. Mol. Biol., _160_,
325-342, 1982). However, the packing together and
orientation of heavy and light chain variable
domains is relatively conserved (Chothia, C.,
Novotny, J., Bruccoleri, R. and Karplus, M., J. Mol.
Biol., _186_, 651-653, 1985). These points will need
to be borne in mind when constructing a new antigen
biding site so as to ensure that packing and
orientation are not altered to the deteriment of
antigen binding capacity.

It is thus clear that merely by replacing one or
more CDRs with complementary CDRs may not always
result in a functional altered antibody. However,
given the explanations set out above, it will be
well within the competence of the man skilled in the
art, either by carrying out routine experimentation
or by trial and error testing to obtain a functional
altered antibody.

Preferably, the variable domains in both the heavy
and light chains have been altered by at least
partial CDR replacement and, if necessary, by
partial framework region replacement and sequence
changing. Although the CDRs may be derived from an
antibody of the same class or even subclass as the
antibody from which the framework regions are
derived, it is envisaged that the CDRs will be
derived from an antibody of different class and
preferably from an antibody from a different
species.

Thus, it is envisaged, for instance, that the CDRs
from a mouse antibody could be grafted onto the

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framework regions of a human antibody. This
arrangement will be of particular use in the
therapeutic use of monoclonal antibodies.

At present, when a mouse monoclonal antibody or even
a chimeric antibody comprising a complete mouse
variable domain is injected into a human, the human
body's immune system recognises the mouse variable
domain as foreign and produces an immune response
thereto. Thus, on subsequent injections of the
mouse antibody or chimeric antibody into the human,
its effectiveness is considerably reduced by the
action of the body's immune system against the
foreign antibody. In the altered antibody of the
present invention, only the CDRs of the antibody
will be foreign to the body, and this should
minimise side effects if used for human therapy.
Although, for example, human and mouse framework
regions have characteristic sequences, there seem
to be no characteristic features which distinguish
human from mouse CDRs. Thus, an antibody comprised
of mouse CDRs in a human framework may well be no
more foreign to the body than a genuine human
antibody.

Even with the altered antibodies of the present
invention, there is likely to be an anti-idiotypic
response by the recipient of the altered antibody.
This response is directed to the antibody binding
region of the altered antibody, It is believed that
at least some anti-idiotype antibodies are directed
at sites bridging the CDRs and the framework regions.
It would therefore be possible to provide a panel of
antibodies having the same partial or complete CDR
replacements but on a series of different framework
regions. Thus, once a first altered antibody became
therapeutically ineffective, due to an anti-idiotype

response, a second altered antibody from the series could be used, and so on, to overcome the effect of the anti-idiotype response. Thus, the useful life of the antigen-binding capacity of the altered antibodies could be extended.

Preferably, the altered antibody has the structure of a natural antibody or a fragment thereof. Thus, the altered antibody may comprise a complete antibody, an (Fab')$_2$ fragment, an Fab fragment, a light chain dimer or a heavy chain dimer. Alternatively, the altered antibody may be a chimeric antibody of the type described in the Neuberger application referred to above. The production of such an altered chimeric antibody can be carried out using the methods described below used in conjunction with the methods described in the Neuberger application.

The present invention, in a second aspect, comprises a method for producing such an altered antibody comprising:

a) preparing a first replicable expression vector including a suitable promoter operably linked to a DNA sequence which encodes at least a variable domain of an Ig heavy or light chain, the variable domain comprising framework regions from a first antibody and CDRs comprising at least parts of the CDRs from a second antibody of different specificity;

b) if necessary, preparing a second replicable expression vector including a suitable promoter operably linked to a DNA sequence which encodes at least the variable domain of a complementary Ig light or heavy chain respectively;

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    c)   transforming a cell line with the first or both prepared vectors; and

    d)   culturing said transformed cell line to produce said altered antibody.

The present invention also includes vectors used to transform the cell line, vectors used in producing the transforming vectors, cell lines transformed with the transforming vectors, cell lines tranformed with preparative vectors, and methods for their production.

Preferably, the cell line which is transformed to produce the altered antibody is an immortalised mammalian cell line, which is advantageously of lymphoid origin, such as a myeloma, hybridoma, trioma or quadroma cell line. The cell line may also comprise a normal lymphoid cell, such as a B-cell, which has been immortalised by transformation with a virus, such as the Epstein-Barr virus. Most preferably, the immortalised cell line is a myeloma cell line or a derivative thereof.

Although the cell line used to produce the altered antibody is preferably a mammalian cell line, any other suitable cell line, such as a bacterial cell line or a yeast cell line, may alternatively be used. In particular, it is envisaged that E. Coli derived bacterial strains could be used.

It is known that some immortalised lymphoid cell lines, such as myeloma cell lines, in their normal state secrete isolated Ig light or heavy chains. If

such a cell line is transformed with the vector
prepared in step a) of the process of the invention,
it will not be necessary to carry out step b) of the
process, provided that the normally secreted chain
is complementary to the variable domain of the Ig
chain encoded by the vector prepared in step a).

However, where the immortalised cell line does not
secrete or does not secrete a complementary chain,
it will be necessary to carry out step b). This
step may be carried out by further manipulating the
vector produced in step a) so that this vector
encodes not only the variable domain of an altered
antibody light or heavy chain, but also the
complementary variable domain.
Alternatively, step b) is carried out by
preparing a second vector which is used to transform
the immortalised cell line. This alternative leads
to easier construct preparation, but may be less
preferred than the first alternative in that it may
not lead to as efficient production of antibody.

The techniques by which such vectors can be produced
and used to transform the immortalised cell lines
are well known in the art, and do not form any part
of the invention.

In the case where the immortalised cell line
secretes a complementary light or heavy chain, the
transformed cell line may be produced for example by
transforming a suitable bacterial cell with the
vector and then fusing the bacterial cell with the
immortalised cell line by spheroplast fusion.
Alternatively, the DNA may be directly introduced
into the immortalised cell line by electroporation.

The DNA sequence encoding the altered variable
domain may be prepared by oligonucleotide
synthesis. This requires that at least the
framework region sequence of the acceptor antibody
and at least the CDRs sequences of the donor
antibody are known or can be readily determined.
Although determining these sequences, the synthesis
of the DNA from oligonucleotides and the preparation
of suitable vectors is to some extent laborious, it
involves the use of known techniques which can
readily be carried out by a person skilled in the
art in light of the teaching given here.

If it was desired to repeat this strategy to insert
a different antigen binding site, it would only
require the synthesis of oligonucleotides encoding
the CDRs, as the framework oligonucleotides can be
re-used.

A convenient variant of this technique would involve
making a symthetic gene lacking the CDRs in which
the four framework regions are fused together with
suitable restriction sites at the junctions. Double
stranded synthetic CDR cassettes with sticky ends
could then be ligated at the junctions of the
framework regions. A protocol for achieving this
variant is shown diagrammatically in Figure 6 of the
accompanying drawings.

Alternatively, the DNA sequence encoding the altered
variable domain may be prepared by primer directed
oligonucleotide site-directed mutagenesis. This

technique in essence involves hybridising an
oligonucleotide coding for a desired mutation with a
single strand of DNA containing the region to be
mutated and using the single strand as a template
for extension of the oligonulcleotide to produce a
strand containing the mutation. This technique, in
various forms, is described by : Zoller, M.J. and
Smith, M., Nuc. Acids Res., 10, 6487-6500, 1982;
Norris, K., Norris F., Christiansen, L. and Fiil,
N., Nuc. Acids Res., 11, 5103-5112, 1983; Zoller,
M.J. and Smith, M., DNA, 3, 479-488 (1984); Kramer,
W., Schughart, K. and Fritz, W.-J., Nuc. Acids Res.,
10, 6475-6485, 1982.

For various reasons, this technique in its simplest
form does not always produce a high frequency of
mutation. An improved technique for introducing
both single and multiple mutations in an M13 based
vector, has been described by Carter et al. (Carter,
P., Bedouelle H. and Winter, G., Nuc. Acids Res.,
13, 4431-4443, 1985)

Using a long oligonucleotide, it has proved possible
to introduce many changes simultaneously (as in
Carter et al., loc. cit.) and thus single
oligonucleotides, each encoding a CDR, can be used
to introduce the three CDRs from a second antibody
into the framework regions of a first antibody. Not
only is this technique less laborious than total
gene synthesis, but it represents a particularly
convenient way of expressing a variable domain of
required specificity, as it can be simpler than
tailoring an entire V_H domain for insertion into an
expression plasmid.

The oligonucleotides used for site-directed
mutagenesis may be prepared by oligonucleotide
synthesis or may be isolated from DNA coding for the
variable domain of the second antibody by use of
suitable restriction enzymes. Such long
oligonucleotides will generally be at least 30 bases
long and may be up to or over 80 bases in length.

The techniques set out above may also be used, where
necessary, to produce the vector of part (b) of the
process.

The method of the present invention is envisaged as
being of particular use in "humanising" non-human
monoclonal antibodies. Thus, for instance, a mouse
monoclonal antibody against a particular human
cancer cell may be produced by techniques well known
in the art. The CDRs from the mouse monoclonal
antibody may then be partially or totally grafted
into the framework regions of a human monoclonal
antibody, which is then produced in quantity by a
suitable cell line. The product is thus a
specifically targetted, essentially human antibody
which will recognise the cancer cells, but will not
itself be recognised to any significant degree, by a
human's immune system, until the anti-idiotype
response eventually becomes apparent. Thus, the
method and product of the present invention will be
of particular use in the clinical environment.

The present invention is now described, by way of
example only, with reference to the accompanying
drawings, in which:

Figure 1 is a schematic diagram showing the structure of an IgG molecule;

Figure 2 shows the amino acid sequence of the V_H domain of NEWM in comparison with the V_H domain of the BI-8 antibody;

Figure 3 shows the amino acid and nucleotide sequence of the HuV_{NP} gene;

Figure 4 shows a comparison of the results for HuV_{NP}-IgE and MoV_{NP}-IgE in binding inhibition assays;

Figure 5 shows the structure of three oligonucleotides used for site directed mutagenesis;

Figure 6 shows a protocol for the construction of CDR replacements by insertion of CDR cassettes into a vector containing four framework regions fused together;

Figure 7 shows the sequence of the variable domain of antibody D1.3 and the gene coding therefor; and

Figure 8 shows a protocol for the cloning of the D1.3 variable domain gene.

EXAMPLE 1

This example shows the production of an altered antibody in which the variable domain of the heavy chains comprises the framework regions of a human heavy chain and the CDRs from a mouse heavy chain.

The framework regions were derived from the human myeloma heavy chain NEWM, the crystallographic structure of which is known (see Poljak et al., loc. cit. and Reth, M., Hammerling, G.J. and Rajewsky, K., EMBO J., 1, 629-634, 1982.)

The CDRs were derived from the mouse monoclonal antibody B1-8 (see Reth et al., loc. cit.), which binds the hapten NP-cap (4-hydroxy-3-nitrophenyl acetyl-caproic acid: $K_{NP-CAP}=1.2$ μM).

A gene encoding a variable domain HuV_{NP}, comprising the B1-8 CDRs and the NEWM framework regions, was constructed by gene synthesis as follows.

The amino acid sequence of the V_H domain of NEWM is shown in Figure 2, wherein it is compared to the amino acid sequence of the V_H domain of the B1-8 antibody. The sequence is divided into framework regions and CDRs according to Kabat et al. (loc. cit.). Conserved residues are marked with a line.

The amino acid and nucleotide sequence of the HuV_{NP} gene, in which the CDRs from the B1-8 antibody alternate with the framework regions of the NEWM antibody, is shown in Figure 3. The HuV_{NP} gene was derived by replacing sections of the MoV_{NP} gene in the vector $pSV-V_{NP}$ (see Neuberger, M.S., Williams, G.T., Mitchell, E.B., Jouhal, S., Flanagan, J.G. and Rabbitts, T.H., Nature, 314, 268-270, 1985) by a synthetic fragment encoding the HuV_{NP} domain. Thus the 5' and 3' non-coding sequences, the leader sequence, the L-V intron, five N-terminal and four

C-terminal amino acids are from the MoV$_{NP}$ gene and the rest of the coding sequence is from the synthetic HuV$_{NP}$ fragment.

The oligonucleotides from which the HuV$_{NP}$ fragment was assembled are aligned below the corresponding portion of the HuV$_{NP}$ gene. For convenience in cloning, the ends of oligonucleotides 25 and 26b form a Hind II site followed by a Hind III site, and the sequences of the 25/26b oligonucleotides therefore differ from the HuV$_{NP}$ gene.

The HuV$_{NP}$ synthetic fragment was built as a PstI-Hind III fragment. The nucleotide sequence was derived from the protein sequence using the computer programme ANALYSEQ (Staden, R., Nuc. Acids. Res., 12, 521-538, 1984) with optimal codon usage taken from the sequences of mouse constant domain genes. The oligonucleotides (1 to 26b, 28 in total) vary in size from 14 to 59 residues and were made on a Biosearch SAM or an Applied Biosystems machine, and purified on 8M-urea polyacrylamide gels (see Sanger, F. and Coulson, A., FEBS Lett., 87, 107-110, 1978).

The oligonucleotides were assembled in eight single stranded blocks (A-D) containing oligonucleotides

[1,3,5,7] (Block A), [2,4,6,8] (block A'), [9,11,13a,13b] (Block B), [10a, 10b,12/14] (block B'), [15, 17] (block C), [16,18] (block C'), [19, 21, 23, 25] (block D) and [20, 22/24, 26a, 26b) (block D').

In a typical assembly, for example of block A, 50
pmole of oligonucleotides 1,3,5 and 7 were
phosphorylated at the 5' end with T4 polynucleotide
kinase and mixed together with 5 pmole of the
terminal oligonucleotide [1] which had been
phosphorylated with 5 μCi [γ-^{32}P] ATP (Amersham 3000
Ci/mmole). These oligonucleotides were annealed by
heating to 80°C and cooling over 30 minutes to room
temperature, with unkinased oligonucleotides 2, 4
and 6 as splints, in 150 μl of 50 mM Tris.Cl, pH
7.5, 10 mM MgCl$_2$. For the ligation, ATP (1 mM) and
DTT (10mM) were added with 50 U T4 DNA ligase
(Anglian Biotechnology Ltd.) and incubated for 30
minutes at room temperature. EDTA was added to 10
mM, the sample was extracted with phenol,
precipitated from ethanol, dissolved in 20 μl water
and boiled for 1 minute with an equal volume of
formamide dyes. The sample was loaded onto and run
on a 0.3 mm 8M-urea 10% polyacrylamide gel. A band
of the expected size was detected by autoradiography
and eluted by soaking.

Two full length single strands were assembled from
blocks A to D and A' to D' using splint
oligonucleotides. Thus blocks A to D were annealed
and ligated in 30 μl as set out in the previous
paragraph using 100 pmole of oligonucleotides 10a,
16 and 20 as splints. Blocks A' to D' were ligated
using oligonucleotides 7, 13b and 17 as splints.

After phenol/ether extraction, block A-D was
annealed with block A'-D', small amounts were cloned
in the vector M13mp18 (Yanish-Perron, C., Vieira, J.
and Messing, J., Gene, 33, 103-119, 1985) cut with
PstI and Hind III, and the gene sequenced by the

dideoxy technique (Sanger, F., Nicklen, S. and Coulson, A.R., PNAS USA, $\underline{74}$, 5463-5467, 1979).

The MoV$_{NP}$ gene was transferred as a Hind III - BamHI fragment from the vector pSV-V$_{NP}$ (Neuberger et al., loc. cit.) to the vector M13mp8 (Messing, J. and Vieira, J., Gene, $\underline{19}$, 269-276, 1982). To facilitate the replacement of MoV$_{NP}$ coding sequences by the synthetic HuV$_{NP}$ fragment, three Hind II sites were removed from the 5' non-coding sequence by site directed mutagenesis, and a new Hind II site was subsequently introduced near the end of the fourth framework region (FR4 in Figure 2). By cutting the vector with PstI and Hind II, most of the V$_{NP}$ fragment can be inserted as a PstI-Hind II fragment. The sequence at the Hind II site was corrected to NEWM FR4 by site directed mutagenesis.

The Hind III - Bam HI fragment, now carrying the HuV$_{NP}$ gene, was excised from M13 and cloned back into pSV-V$_{NP}$ to replace the MoV$_{NP}$ gene and produce a vector pSV-HuV$_{NP}$. Finally, the genes for the heavy chain constant domains of human Ig E (Flanagan, J.G. and Rabbitts, T.H., EMBO J., $\underline{1}$, 655-660, 1982) were introduced as a Bam HI fragment to give the vector pSV-HuV$_{NP}$. HE. This was transfected into the myeloma line J558 L by spheroplast fusion.

The sequence of the HuV$_{NP}$ gene in pSV-HuV$_{NP}$. HE was checked by recloning the Hind III-Bam HI fragment back into M13mp8 (Messing et al., loc. cit.). J558L myeloma cells secrete lambda 1 light chains which have been shown to associate with heavy chains containing the MoV$_{NP}$ variable domain to create a

binding site for NP-cap or the related hapten
NIP-Cap (3-iodo-4-hydroxy-5-nitrophenylacetyl-
caproic acid) (Reth, M., Hammerling, G.J. and
Rajewsky, K., Eur. J. Immunol., 8, 393-400, 1978).

As the plasmid pSV-HuV$_{NP}$.HE contains the gpt marker,
stably transfected myeloma cells could be selected
in a medium containing mycophenolic acid.
Transfectants secreted an antibody (HuV$_{NP}$-IgE) with
heavy chains comprising a HuV$_{NP}$ variable domain
(i.e. a "humanised" mouse variable region) and
human γ constant domains, and lambda 1 light chains
from the J558L myeloma cells.

The culture supernatants of several gpt[+] clones were
assayed by radioimmunoassay and found to contain
NIP-cap binding antibody. The antibody secreted by
one such clone was purified from culture supernatant
by affinity chromatography on NIP-cap Sepharose
(Sepharose is a registered trade mark). A
polyacrylamide - SDS gel indicated that the protein
was indistinguishable from the chimeric antibody
MoV$_{NP}$-IgE (Neuberger et al., loc. cit.).

The HuV$_{NP}$-IgE antibody competes effectively with the
MoV$_{NP}$-IgE for binding to both anti-human-IgE and to
NIP-cap coupled to bovine serum albumin.

Various concentrations of HuV$_{NP}$-IgE and MoV$_{NP}$-IgE
were used to compete the binding of radiolabelled
MoV$_{NP}$-IgE to polyvinyl microtitre plates coated with
(a) Sheep anti-human-IgE antiserum (Seward
Laboratories); (b) NIP-cap-bovine serum albumin; (c)
Ac38 anti-idiotypic antibody; (d) Ac 146
anti-idiotypic antibody; and (e) rabbit anti-MoV$_{NP}$

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antiserum. Binding was also carried out in the
presence of MoV$_{NP}$-IgM antibody (Neuberger, M.S.,
Williams, G.T. and Fox, R.O., Nature, 312, 604-608,
1984) or of JW5/1/2 which is an IgM antibody
differing from the MoV$_{NP}$-IgM antibody at 13 residues
mainly located in the V$_H$ CDR2 region.

The results of the binding assays are shown in
Figure 4, wherein black circles represent HuV$_{NP}$,
white circles MoV$_{NP}$, black squares MoV$_{NP}$-IgM and
white squares JW5/1/2. Binding is given relative to
the binding in the absence of the inhibitor.

The affinities of HuV$_{NP}$-IgE for NP-cap and NIP-cap
were then measured directly using the fluorescence
quench technique and compared to those for
MoVNP-IgE, using excitation at 295 nm and observing
emission at 340 nm (Eisen, H.N., Methods Med. Res.,
10, 115-121,1964).

Antibody solutions were diluted to 100 nM in
phosphate buffered saline, filtered (0.45 μm pore
cellulose acetate) and titrated with NP-cap in the
range 0.2 to 20 μM. As a control, mouse DI-3
antibody (Mariuzza, R.A., Jankovic, D.L., Bulot, G.,
Amit, A.G., Saludjian, P., Le Guern, A., Mazie, J.C.
and Poljak, R.J., J. Mol. Biol., 170, 1055-1058,
1983), which does not bind hapten, was titrated in
parallel.

Decrease in the ratio of the fluorescence of
HuV$_{NP}$-IgE or HuV$_{NP}$-IgE to the fluorescence of the
D1-3 antibody was taken to be proportional to NP-cap
occupancy of the antigen binding sites. The maximum

quench was about 40% for both antibodies, and hapten
dissociation constants were determined from
least-squares fits of triplicate data sets to a
hyperbola.

For NIP-cap, hapten concentration varied from 10 to
300 nM, and about 50% quenching of fluorescence was
observed at saturation. Since the antibody
concentrations were comparable to the value of the
dissociation constants, data were fitted by least
squares to an equation describing tight binding
inhibition (Segal, I.H., in "Enzyme Kinetics",
73-74, Wiley, New York, 1975).

The binding constants obtained from these data for
these antibodies are shown in Table 1 below.

Table 1

	K_{NP-cap}	$K_{NIP-cap}$
$MoV_{NP}-IgE$	1.2 μM	0.02 μM
$HuV_{NP}-IgE$	1.9 μM	0.07 μM

These results show that the affinities of these
antibodies are similar and that the change in
affinity is less than would be expected for the loss
of a hydrogen bond or a van der Waals contact point
at the active site of an enzyme.

Thus, it has been shown that it is possible to
produce an antibody specific for an artificial small
hapten, comprising a variable domain having human
framework regions and mouse CDRs, without any
significant loss of antigen binding capacity.

As shown in Figure 4(d), the HuV_{NP}-IgE antibody has lost the MoV_{NP} idiotypic determinant recognised by the antibody Ac146. Furthermore, HuV_{NP}-IgE also binds the Ac38 antibody less well (Figure 4(c)), and it is therefore not surprising that HuV_{NP}-IgE has lost many of the determinants recognised by the polyclonal rabbit anti-idiotypic antiserum (Figure 4(e)).

It can thus be seen that, although the HuV_{NP}-IgE antibody has acquired substantially all the antigen binding capacity of the mouse CDRs, it has not acquired any substantial proportion of the mouse antibody's antigenicity.

The results of Figures 4(d) and 4(e) carry a further practical implication. The mouse (or human) CDRs could be transferred from one set of human frameworks (antibody 1) to another (antibody 2). In therapy, anti-idiotypic antibodies generated in response to antibody 1 might well bind poorly to antibody 2. Thus, as the anti-idiotypic response starts to neutralise antibody 1 treatment could be continued with antibody 2, and the CDRs of a desired specificity used more than once.

For instance, the oligonucleotides encoding the CDRs may be used again, but with a set of oligonucleotides encoding a different set of framework regions.

The above work has shown that antigen binding characteristics can be transferred from one framework to another without loss of activity, so

long as the original antibody is specific for a
small hapten.

It is known that small haptens generally fit into an
antigen binding cleft. However, this may not be
true for natural antigens, for instance antigens
comprising an epitopic site on a protein or
polysaccharide. For such antigens, the antibody
may lack a cleft (it may only have a shallow
concavity), and surface amino acid residues may play
a significant role in antigen binding. It is
therefore not readily apparent that the work on
artificial antigens shows conclusively that CDR
replacement could be used to transfer natural
antigen binding properties.

Therefore work was carried out to see if CDR
replacement could be used for this purpose. This
work also involved using primer-directed,
oligonucleotide site-directed mutagenesis using
three synthetic oligonucleotides coding for each of
the mouse CDRs and the flanking parts of framework
regions to produce a variable domain gene similar to
the HuV_{NP} gene.

EXAMPLE 2

The three dimensional structure of a complex of
lysozyme and the antilysozyme antibody D1.3 (Amit et
al., loc. cit.) was solved by X-ray
crystallography. There is a large surface of
interaction between the antibody and antigen. The
antibody has two heavy chains of the mouse IgG1
class (H) and two Kappa light chains (K), and is
denoted below as H_2K_2.

The DNA sequence of the heavy chain variable
region was determined by making cDNA from the mRNA
of the D1.3 hybridoma cells, and cloning into
plasmid and M13 vectors. The sequence is shown in
Figure 7, in which the boxed residues comprise the
three CDRs and the asterisks mark residues which
contact lysozyme.

Three synthetic oligonucleotides were then designed
to introduce the D1.3 V_HCDRs in place of the V_HCDRs
of the HuV_{NP} gene. The Hu_{NP} gene has been cloned
into M13mp8 as a BamHI-Hind III
fragment, as described above. Each oligonucleotide
has 12 nucleotides at the 5' end and 12 nucleotides
at the 3' end which are complementary to the
appropriate HuV_{NP} framework regions. The central
portion of each oligonucleotide encodes either CDR1,
CDR2, or CDR3 of the D1.3 antibody, as shown in
Figure 5, to which reference is now made. It can be
seen from this Figure that these oligonucleotides
are 39, 72 and 48 nucleotides long respectively.

10 pmole of D1.3 CDR1 primer was phosphorylated at
the 5' end and annealed to 1µg of the M13-HuV_{NP}
template and extended with the Klenow fragment of
DNA polymerase in the presence of T4 DNA ligase.
After an oligonucleotide extension at 15°C, the
sample was used to transfect E. Coli strain BHM71/18
mutL and plaques gridded and grown up as infected
colonies.

After transfer to nitrocellulose filters, the
colonies were probed at room temperature with 10
pmole of D1.3 CDR1 primer labelled at the 5' end

with 30 μCi^{32}-p-ATP. After a 3" wash at 60°C,
autoradiography revealed about 20% of the colonies
had hybridised well to the probe. All these
techniques are fully described in "Oligonucleotide
site-directed mutagenesis in M13" an experimental
manual by P. Carter, H. Bedouelle, M.M.Y. Waye and
G. Winter 1985 and published by Anglian
Biotechnology Limited, Hawkins Road, Colchester,
Essex CO2 8JX. Several clones were sequenced, and
the replacement of HuV_{NP} CDR1 by D.13 CDR1 was
confirmed. This M13 template was used in a second
round of mutagenesis with D1.3 CDR2 primer; finally
template with both CDRs 1&2 replaced was used in a
third round of mutagenesis with D.13 CDR3 primer.
In this case, three rounds of mutagenesis were
used.   .

The variable domain containing the D1.3 CDRs was
then attached to sequences encoding the heavy chain
constant regions of human IgG2 so as to produce a
vector encoding a heavy chain Hu*. The vector was
transfected into J558L cells as above. The antibody
$Hu*_2L_2$ is secreted.

For comparative purposes, the variable region gene
for the D1.3 antibody was inserted into a suitable
vector and attached to a gene encoding the constant
regions of mouse IgG1 to produce a gene encoding a
heavy chain H* with the same sequence as H. The
protocol for achieving this is shown in Figure 8.

As shown in Figure 8, the gene encoding the D1.3
heavy chain V and C_H1 domains and part of the hinge
region are cloned into the M13mp9 vector.

The vector (vector A) is then cut with NcoI, blunted
with Klenow polymerase and cut with PstI. The
PStI-NcoI fragment is purified and cloned into
PstI-HindII cut MV_{NP} vector to replace most of the
MV_{NP} coding sequences. The MV_{NP} vector comprises
the mouse variable domain gene with its promoter, 5'
leader, and 5' and 3' introns cloned into M13mp9.
This product is shown as vector B in Figure 8.

Using site directed mutagenesis on the single
stranded template of vector B with two primers, the
sequence encoding the N-terminal portion of the C_H1
domain and the PstI site near the N-terminus of the
V domain are removed. Thus the V domain of Dl.3 now
replaces that of V_{NP} to produce vector C of Figure 8.

Vector C is then cut with HindIII and BamHI and the
fragment formed thereby is inserted into
HindIII/BamHI cut M13mp9. The product is cut with
Hind III and SacI and the fragment is inserted into
PSV-V_{NP} cut with Hind III/SacI so as to replace the
V_{NP} variable domain with the Dl.3 variable domain.
Mouse IgG1 constant domains are cloned into the
vector as a SacI fragment to produce vector D of
Figure 8.

Vector D of Figure 8 is transfected into J558L cells
and the heavy chain H* is secreted in association
with the lambda light chain L as an antibody $H*_2L_2$.

Separated K or L light chains can be produced by
treating an appropriate antibody (for instance Dl.3
antibody to produce K light chains) with
2-mercaptoethanol in guanidine hydrochloride,

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blocking the free interchain sulphydryls with
iodoacetamide and separating the dissociated heavy
and light chains by HPLC in guanidine hydrochloride.

Different heavy and light chains can be reassociated
to produce functional antibodies by mixing the
separated heavy and light chains, and dialysing into
a non-denaturing buffer to promote re-association
and refolding. Properly reassociated and folded
antibody molecules can be purified on protein
A-sepharose columns. Using appropriate combinations
of the above procedures, the following antibodies
were prepared.

H_2K_2 (Dl 3 antibody)
$H^*_2L_2$ (Dl.3 heavy chain, lambda light chain)
$H^*_2K_2$ (recombinant equivalent of Dl.3)
$Hu^*_2L_2$ ("humanised" Dl.3 heavy chain, lambda
                  light chain)
$Hu^*_2K_2$ ("humanised" Dl.3)

The antibodies containing the lambda light chains
were not tested for antigen binding capacity. The
other antibodies were, and the results are shown in
Table 2.

Table 2

Antibody	Dissociation constant for lysozyme (nM)
Dl.3 (H_2K_2)	14.4
Dl.3 (H_2K_2) (reassociated)	15.9, 11.4

recombinant D1.3 (H$^*_2$K$_2$)   9.2
(reassociated)

"humanised" D1.3 (Hu$_2$K$_2$)   3.5, 3.7
(reassociated)

The affinity of the antibodies for lysozyme was
determined by fluorescent quenching, with excitation
at 290nm and emission observed at 340nm Antibody
solutions were diluted to 15-30µg/mg in phosphate
buffered saline, filtered (0.45 um-cellulose
acetate) and titrated with hen eggwhite lysozyme.
There is a quenching of fluorescence on adding the
lysozyme to the antibody (>100% quench) and data
were fitted by least squares to an equation
describing tight binding inhibition (I.H. Segal in
Enzyme Kinetics, p73-74, Wiley, New York 1975).
Although at first sight the data suggest that the
binding of the "humanised" antibody to lysozyme is
tighter than in the original D1.3 antibody, this
remains to be confirmed. It is clear however that
the humanised antibody binds lysozyme with a
comparable affinity to D1.3

Further work (with another antibody-CAMPATH1) has
shown that CDRs 1,2 and 3 can be exchanged
simultaneously, by priming as above with all three
primers. 10% hybridisation positives were detected
by screening with the CDR1 primer; 30% of these
comprised the triple mutant in which all the CDRs
were replaced.

It has therefore been shown that CDR replacement can be used not only for artificial antigens (haptens) but also for natural antigens, thereby showing that the present invention will be of therapeutic use.

It will of course be understood that the present invention has been described above purely by way of example, and modifications of detail can be made within the scope of the invention as defined in the appended claims.

CLAIMS

1.   An altered antibody in which at least parts of
the complementarity determining regions (CDRs) in
the light or heavy chain variable domains have been
replaced by analogous parts of CDRs from an antibody
of different specificity.

2.   The altered antibody of claim 1, in which the
entire CDRs have been replaced.

3.   The altered antibody of claim 1 or claim 2, in
which the variable domains in both the heavy and
light chains have been altered by CDR replacement.

4.   The altered antibody of any one of claims 1 to
3 in which the CDRs from a mouse antibody are
grafted onto the framework regions of a human
antibody.

5.   The altered antibody of any one of claims 1
to 4, which has the structure of a natural antibody
or a fragment thereof.

6.   A method for producing an altered antibody
comprising:

    a)   preparing a first replicable expression
vector including a suitable promoter operably linked
to a DNA sequence which encodes at least a variable
domain of an Ig heavy or light chain, the variable
domain comprising framework regions from a first
antibody and CDRs comprising at least parts of the
CDRs from a second antibody of different specificity;

b)   if necessary, preparing a second replicable expression vector including a suitable promoter operably linked to a DNA sequence which encodes at least the variable domain of a complementary Ig light or heavy chain respectively;

c)   transforming a cell line with the first or both prepared vectors; and

d)   culturing said transformed cell line to produce said altered antibody.

7.   The method of claim 6, in which the cell line which is transformed to produce the altered antibody is an immortalised mammalian cell line.

8.   The method of claim 7, in which the immortalised cell line is a myeloma cell line or a derivative thereof.

9.   The method of any one of claims 6 to 8, in which the DNA sequence encoding the altered variable domain is prepared by oligonucleotide synthesis.

10.   The method of any one of claims 6 to 8, in which the DNA sequence encoding the altered variable domain is prepared by primer directed oligonucleotide site-directed mutagenesis using a long oligonucleotide.

Fig. 1
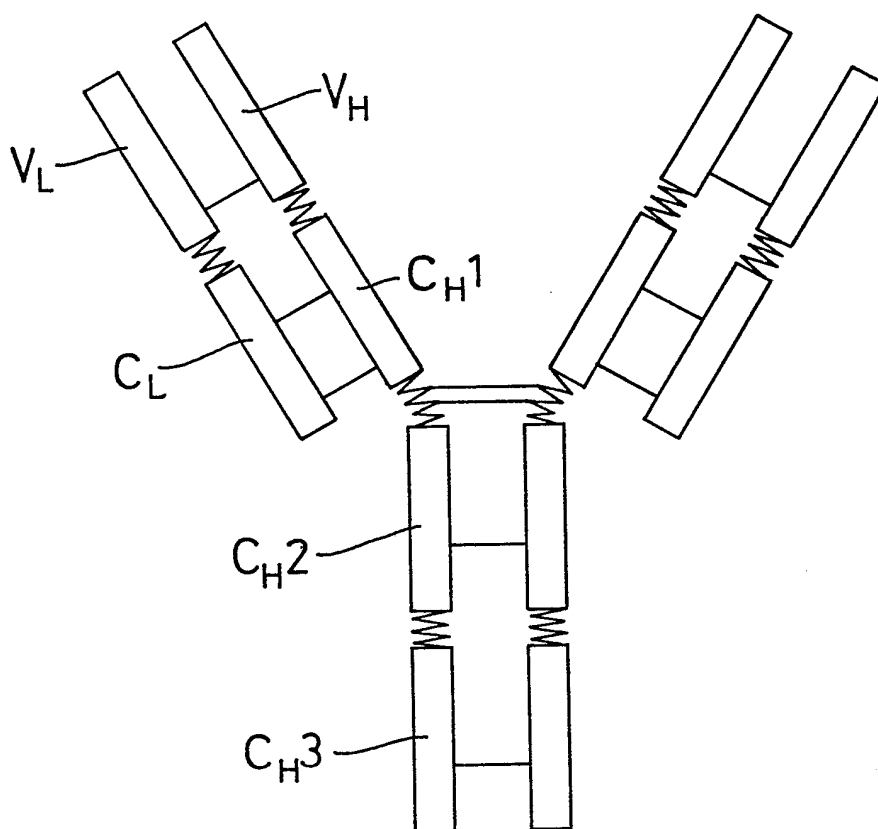
▭	=	domains
∿∿	=	inter-domain sections
—	=	disulphide bonds
V	=	variable
C	=	constant
L	=	light chain
H	=	heavy chain

FR1
```
        1                              30
NEWM    XVQLQESGPGLVRPSQTLSLTCTVSGGSTFS
B1-8    QVQLQQPGAELVKPGASVKLSCKASGYTFT
```

CDR1
```
        31    35
NEWM    NDYYT
B1-8    SYWMH
```

FR2
```
        36           49
NEWM    WVRQPPGRGLEWIG
B1-8    WVKQRPGRGLEWIG
```

CDR2
```
        50              65
NEWM    YVFYHGTSDDTTPLRS
B1-8    RIDPNSGGTKYNEKFKS
```

FR3
```
        66                              94
NEWM    RVTMLVDTSKNQFSLRLSSVTAADTAVYYCAR
B1-8    KATLTVDKPSSTAYMQLSSLTSEDSAVYYCAR
```

CDR3
```
        95        102
NEWM    NLIAGCIDV
B1-8    YDYYGSSYFDY
```

FR4
```
        103        113
NEWM    WGQGSLVTVSS
B1-8    WGQGTTLTVSS
```

Fig. 2

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HindIII -48   -23   -7
↓
5'···········ATGCAAATCCTCTGAATCTACATGGTAAATATAGGTTTGTCTATAC

■→ RNA starts   ■→ RNA starts
CACAAACAGAAAAACATGAGATCACAGTTCTCTCTACAGTTACTGAGCACACAGGACCTC

NP leader   Splice
M G W S C I I L F L V A T A T ↓
ACCATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGTAAGGGGCTC

ACAGTAGCAGGCTTGAGGTCTGGACATATATATGGGTGACAATGACATCCACTTTGCCTT

Splice   1   5 PstI   10
↓G V H S Q V Q L Q↓E S G P G L V R
TCTCTCCACAGGTGTCCACTCCCAGGTCCAACTGCA GGAGAGCGGTCCAGGTCTTGTGAG
                                  5'└────────1────────
                            3'┌────────2────────┐ ┌──

15   20   25   30   CDR1
P S Q T L S L T C T V S G S T F S [S Y W]
ACCTAGCCAGACCCTGAGCCTGACCTGCACCGTGTCTGGCAGCACCTTCAGCAGCTACTG
┘└─────3─────┘ └─────5─────┘ └─────8─────
──────4──────┐ ┌─────6─────┐ ┌─────7

35   40   45   50 CDR2 52A
[M H] W V R Q P P G R G L E W I G [R I D P]
GATGCACTGGGTGAGACAGCCACCTGGACGAGGTCTTGAGTGGATTGGAAGGATTGATCC
──────7──────┘ └─────9a─────┘ └────9b────┘ └────11────
──────┐ ┌─────10a─────┐ ┌────10b────┐ ┌────12/14────

55   CDR2   60   65   70
[N S G G T K Y N E K F K S] R V T M L V D
TAATAGTGGTGGTACTAAGTACAATGAGAAGTTCAAGAGCAGAGTGACAATGCTGGTAGA
────11────┘ └───13a───┘ └───13b───┘ └───15───
──────12/14──────┘   ┌────16────

75   80   82A B C   85
T S K N Q F S L R L S S V T A A D T A V
CACCAGCAAGAACCAGTTCAGCCTGAGACTCAGCAGCGTGACAGCCGCCGACACCGCGGT
────15────┘ └────17────┘ └────19────
──────┐ ┌──────18──────┐ ┌──────20──────

90   95 CDR3   100A B C   105
Y Y C A R [Y D Y Y G S S Y F D Y] W G Q G
CTATTATTGTGCAAGATACGATTACTACGGTAGTAGCTACTTTGACTACTGGGGTCAAGG
────19────┘ └──────21──────┘ └──────23──────┘ └──
──────┐ ┌──────22/24──────┐ ┌──26a──

110   Splice   BamHI
S L V T V S S ↓   ↓
CAGCCTCGTCACAGTCTCCTCAGGT·······193bp·····3'
──25────GACA 3'
┐ ┌─26b─CTGTTCGA 5'

Fig. 3

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Fig. 4

5/?

Fig. 5

HindIII   1   5'   2   5'   3   5'   BamHI

FR1   FR2   FR3   FR4

CDR1   CDR2   CDR3

HuV_{NP} gene cloned in M13mp8

D1.3 CDR1 oligonucleotide
5' CTG,TCT,CAC,CCA,GTT,TAC,ACC,ATA,GCC,GCT,GAA,GGT,GCT

     FR2   D1.3 CDR1   FR1

D1.3 CDR2 oligonucleotide
5' CAT,TGT,CAC,TCT,GGA,TTT,GAG,AGC,TGA,ATT,ATA,GTC,TGT,

     FR3   D1.3 CDR2
GTT,TCC,ATC,ACC,CCA,AAT,CAT,TCC,AAT,CCA,CTC

   D1.3 CDR2   FR2

D1.3 CDR3 oligonucleotide
5' GCC,TTG,ACC,CCA,GTA,GTC,AAG,CCT,ATA,ATC,TCT,CTC,TCT,

     FR4   D1.3 CDR3

TGC,ACA,ATA

   FR3

Fig. 6.

RESTRICTION SITES

1   2   3

VECTOR   FR1   FR2   FR3   FR4   VECTOR

(1) Digest vector/FR1-4 at restriction
    sites 1, 2, & 3.
(2) Ligate CDR sticky end duplexes 1, 2 & 3.

═════ CDR1   +   ═════ CDR2

+   ═════ CDR3

VECTOR   FR1   CDR1   FR2   CDR2   FR3   CDR3   FR4   VECTOR

Fig. 7.

```
              -15  SIGNAL        -10                    -5
             ┌ M  A  V  L  A  L  L  F  C  L  V  T  F  P  S  C  I  L ┐
    TCAGAGCATGGCTGTCCTGGCATTACTCTTCTGCCTGGTAACATTCCCAAGCTGTATCCT
       -1 +1           5              10              15
             ┌ S ┐ Q  V  Q  L  K  E  S  G  P  G  L  V  A  P  S  Q  S  L  S
    TTCCCAGGTGCAGCTGAAGGAGTCAGGACCTGGCCTGGTGGCGCCCTCACAGAGCCTGTC
             [A]
     20              25                    *   *   *  CDR1  35
      I   T  C  T  V  S  G  F  S  L  T ┌ G  Y  G  V  N ┐ W  V  R  Q
    CATCACATGCACCGTCTCAGGGTTCTCATTAACCGGCTATGGTGTAAACTGGGTTCGCCA

     40              45                    50      *   *   * 55      CDR2
      P   P  G  K  G  L  E  W  L  G ┌ M  I  W  G  D  G  N  T  D  Y ┐
    GCCTCCAGGAAAGGGTCTGGAGTGGCTGGGAATGATTTGGGGTGATGGAAACACAGACTA

     60    CDR2    65                    70              75
     ┌ N   S  A  L  K  S ┐ R  L  S  I  S  K  D  N  S  K  S  Q  V  F
    TAATTCAGCTCTCAAATCCAGACTGAGCATCAGCAAGGACAACTCCAAGAGCCAAGTTTT
     80     82A B  C  I      85              90              95  *
      L   K  M  N  S  L  H  T  D  D  T  A  R  Y  Y  C  A  R ┌ E  R ┐
    CTTAAAAATGAACAGTCTGCACACTGATGACACAGCCAGGTACTACTGTGCCAGAGAGAG
                                                          ←
        *   *   *  CDR3    105              110
     ┌ D   Y  R  L  D  Y ┐ W  G  Q  G  T  T  L  T  V  S  S
    AGATTATAGGCTTGACTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCA
    ──── D ───→←─────────────── J_H 2 ──────────────→
```

Fig. 8.

A

	V domain		CH1 domain	hinge

H3 P B   P   N   E

VNP promoter
leader, N-term

VNP
C-term,
3' intron

B

L   V domain

H3   P   N   B E

VNP promoter
leader, N-term

VNP
C-term,
3' intron

C

L   V domain

H3   B E

VNP promoter
leader, N-term

VNP
C-term,
3' intron

mouse IgG1 genomic clone

D

L   V domain   CH1   hinge   CH2   CH3

H3   S   S B
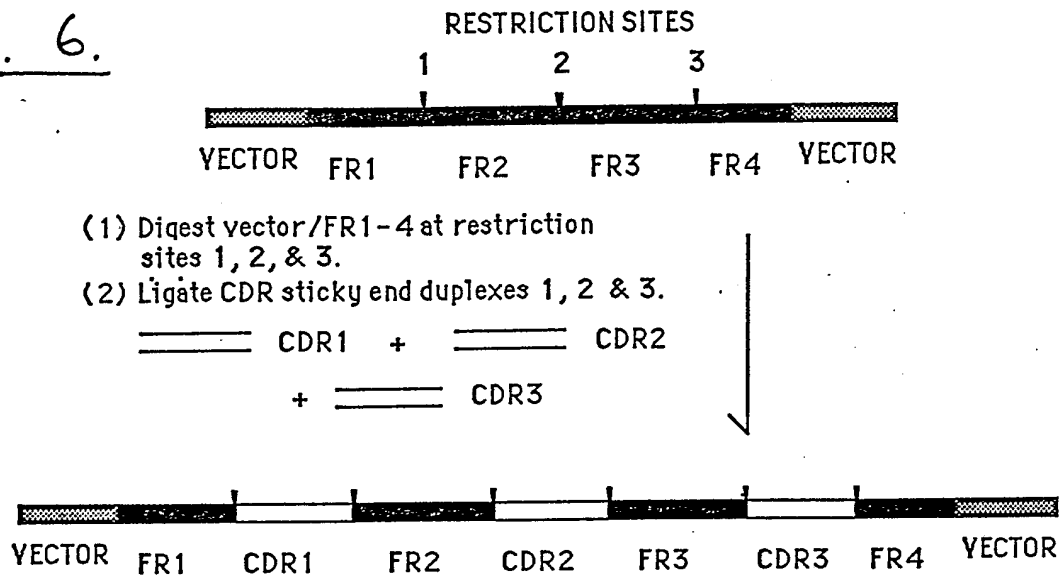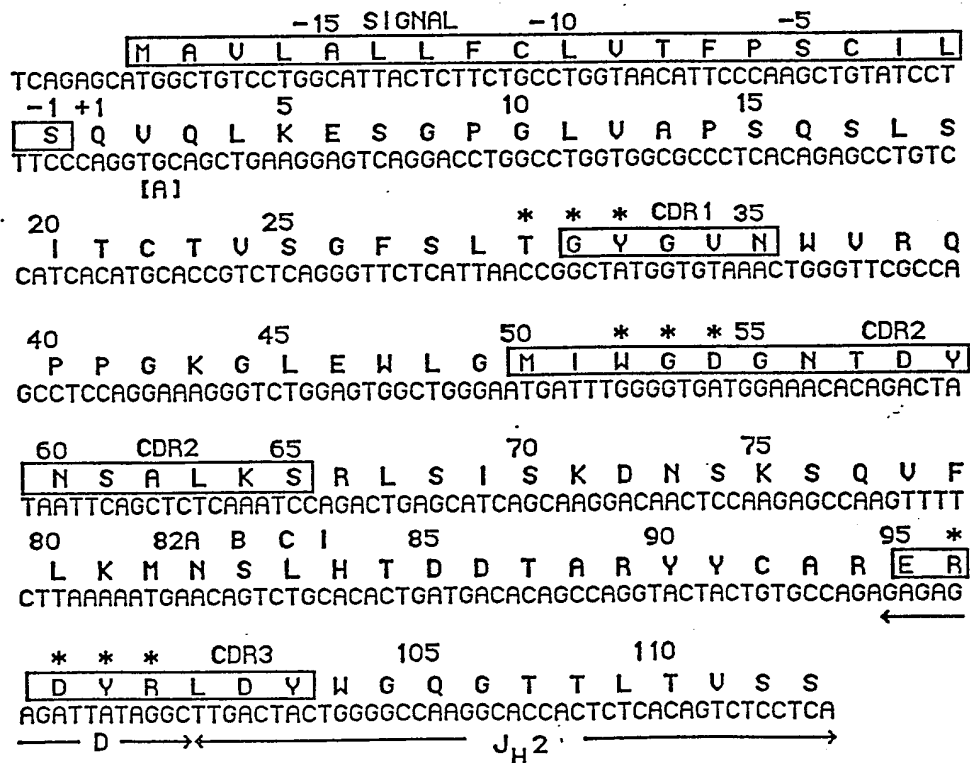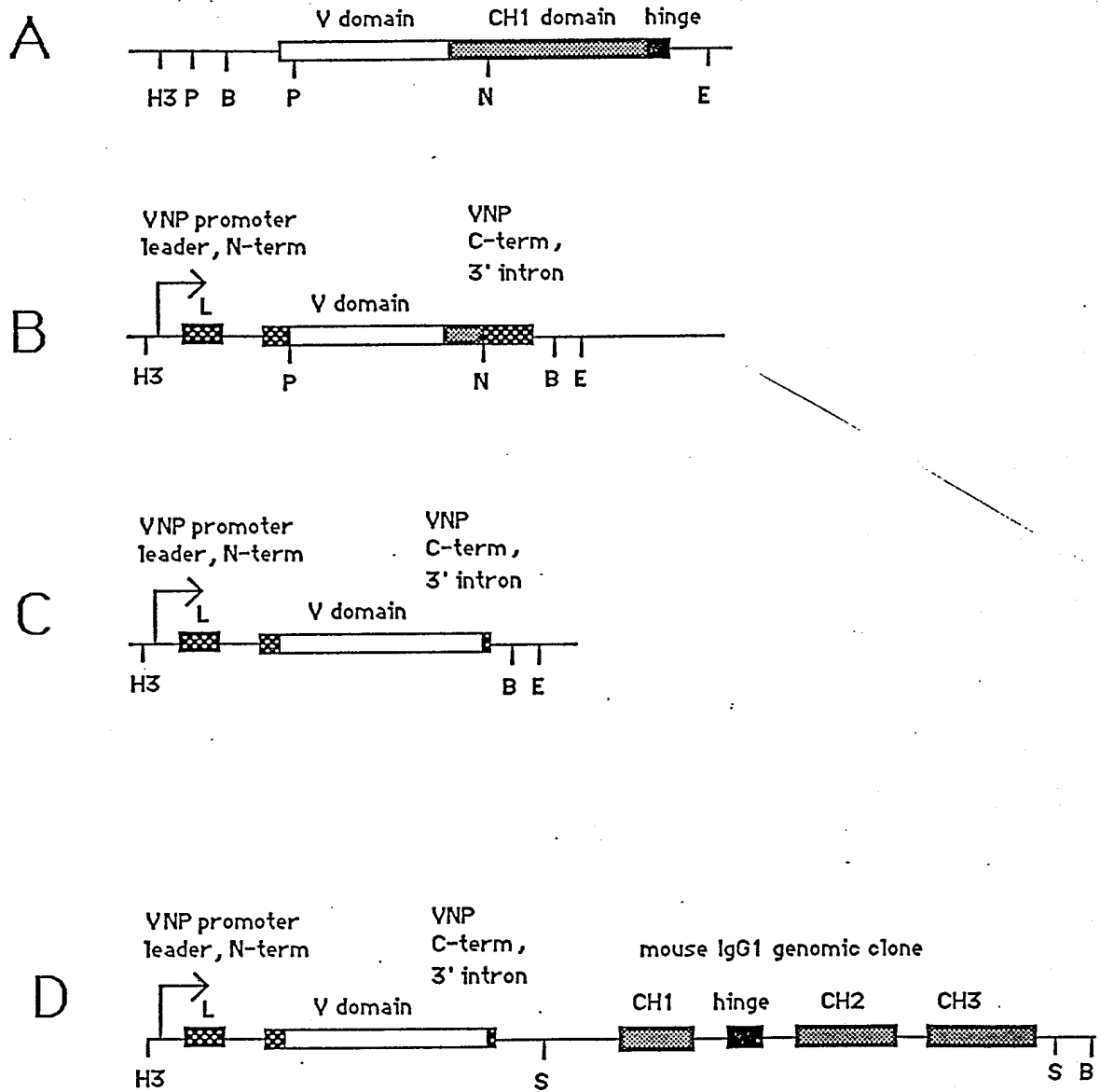
H3 = HindIII, P = PstI, B = BamHI, N = Ncol, E = EcoRI, H2 = HindII